Influence of 7,8-methylenedioxylycoctonine–type alkaloids on the toxic effects associated with ingestion of tall larkspur (*Delphinium* spp) in cattle

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Objective—To determine the contribution of 7,8-methylenedioxylycoctonine (MDL)–type alkaloids to the toxic effects of tall larkspur (*Delphinium* spp) consumption in cattle.

Animals—Sixteen 2-year-old Angus steers.

Procedures—Plant material from 3 populations of tall larkspur that contained different concentration ratios of MDL-type-to-*N*-(methylsuccinimido) anthranoyllycoctonine (MSAL)–type alkaloids was collected, dried, and finely ground. For each plant population, a dose of ground plant material that would elicit similar clinical signs of toxicosis in cattle after oral administration was determined on the basis of the plants' MSAL-type alkaloid concentration. Cattle were treated via oral gavage with single doses of ground plant material from each of the 3 populations of tall larkspur; each animal underwent 1 to 3 single-dose treatments (≥ 21-day interval between treatments). Heart rate was recorded immediately before (baseline) and 24 hours after each larkspur treatment.

Results—Tall larkspur populations with a lower MDL-type-to-MSAL-type alkaloid concentration ratio required a greater amount of MSAL-type alkaloids to cause the expected clinical signs of toxicosis (including increased heart rate) in cattle.

Conclusions and Clinical Relevance—Results indicated that the typically less toxic MDL-type alkaloids contributed in a significant manner to the toxic effects of tall larkspur in steers. Consequently, both the concentration of MSAL-type alkaloids and the total concentration of MSAL- and MDL-type alkaloids should be determined when assessing the relative toxicity of tall larkspur populations. These results provide valuable information to determine the risk of toxicosis in cattle grazing on tall larkspur–infested rangelands. (*Am J Vet Res* 2010;71:487–492)

Larkspurs (*Delphinium* spp) pose one of the most serious poisonous plant problems on foothill and mountain rangelands in the western United States.[1–3] In areas where these plants grow in abundance, mortality rate in cattle attributable to poisoning via ingestion of toxic tall larkspur species is estimated to be between 5% and 15% annually.[1] The annual total cost to the livestock industry of cattle deaths as a result of tall larkspur poisoning is estimated to be in the order of millions of dollars annually.[4] The toxic effects of tall larkspur are attributable to norditerpenoid alkaloids, which occur as 1 of 2 chemical structural types: the MDL-type (including deltaline) and the MSAL-type (including MLA).[1] Although the MSAL-type alkaloids are more toxic (typically > 20 times as toxic),[5,6] the MDL-type alkaloids are generally more abundant in *Delphinium barbeyi* and *Delphinium occidentale* populations.[1,7] On the basis of the MSAL-type alkaloid content, *Delphinium glaucum* is considered the most toxic tall larkspur species, followed by *D barbeyi* and *Delphinium glaucescens*, with *D occidentale* being the least toxic of all tall larkspur species.[8]

Management recommendations for cattle grazing on tall larkspur–infested ranges are primarily based on the concentration of the MSAL-type alkaloids in the tall larkspur plants.[9,10] However, research[11] in mice revealed that the MDL-type alkaloids impart an additive effect on the toxic effects caused by MLA. The purpose of the study reported here was to determine the con-

ABBREVIATIONS

FTIR	Fourier transform infrared
MDL	7,8-methylenedioxylycoctonine
MH^+	Protonated molecular ion
MLA	Methyllycaconitine
MSAL	*N*-(methylsuccinimido) anthranoyllycoctonine
m/z	Mass-to-charge ratio

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tribution of MDL-type alkaloids to the toxic effects of tall larkspur consumption in cattle. The hypothesis was that if MDL-type alkaloids affect the toxicity of the tall larkspur plant material in an additive manner, then a tall larkspur population with a high MDL-type alkaloid concentration would be more toxic than a tall larkspur population with a low MDL-type alkaloid concentration but similar MSAL-type alkaloid content.

Materials and Methods

Plant material—Tall larkspur (*D barbeyi*) in the flowering stage was collected during July 2003 at an elevation of approximately 3,000 m near Manti, Utah (N lat 39°03.154′, W long 111°30.752′; Poisonous Plant Research Laboratory collection No. 03-12). *Delphinium barbeyi* in the flowering stage was also collected during July 2007 at an elevation of approximately 3,300 m near Cedar City, Utah (N lat 37°40.223′, W long 112°49.335′; Poisonous Plant Research Laboratory collection No. 07-06). *Delphinium glaucescens* in the flowering stage was collected during July 2008 at an elevation of approximately 2,500 m near Dillon, Mont (N lat 45°25.888′, W long 112°42.524′; Poisonous Plant Research Laboratory collection No. 08-07). For purposes of the study, these plant collections were designated as the Manti, Cedar City, and Dillon collections.

The plant material from each collection was air-dried, ground until sufficiently fine to pass through a 2.4-mm mesh screen, and mixed by use of a grinder-mixer[a] to provide 1 homogenous lot for each collection. After processing, the ground plant material was stored in plastic bags away from direct light in an enclosed, nonheated, uninsulated building at ambient temperature until alkaloid analysis.

Alkaloid analyses—Each plant collection was analyzed for alkaloid content immediately prior to the start of this study. Protonated molecular ion adduct profiles of plant extracts were acquired to confirm the expected presence of the MSAL- and MDL-type alkaloids by use of an electrospray ionization mass spectrometry method as described.[12] The alkaloidal extracts of the plants were also quantitatively analyzed for MDL-type, MSAL-type, and total alkaloid contents by use of an FTIR spectroscopy method.[13] By use of the FTIR spectroscopy method, only the amount of total alkaloids and MSAL-type alkaloids were directly quantitated. The amount of MDL-type alkaloids was determined by subtracting the amount of MSAL-type alkaloids from the amount of total alkaloids. Values derived via the FTIR spectroscopy method represented the amount (mg) of alkaloid per gram of dried plant material.

Animals—Sixteen 2-year-old Angus steers (mean ± SD weight, 492 ± 28 kg) were fed alfalfa-grass hay and a dietary mineral supplement (a standard diet provided to all cattle housed at the Poisonous Plant Research Laboratory). All steers were acclimated to handling and restraint in a squeeze chute prior to the start of the experiment to minimize stress during handling and physiologic monitoring. Feed was withheld for approximately 18 hours prior to the start of the experiment. All animal experiments were completed under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee.

Dose administrations and monitoring procedures—At the start of the experiment, steers were weighed and then restrained in a squeeze chute. Baseline physiologic measurements of each steer were recorded immediately prior to the administration of a single dose of tall larkspur; the dose was determined on the basis of the MSAL-type alkaloid concentration (mg of MSAL-type alkaloids/kg) determined via FTIR spectroscopy analysis. Each dose of the dried, finely ground tall larkspur was administered via oral gavage in approximately 8 L of tap water. After treatment, the steers were monitored for 48 hours for a decrease in gastrointestinal motility, shuffling gait, development of muscle weakness and trembling, and collapse. Twenty-four hours after dose administration, each steer was again restrained in a squeeze chute and physiologic measurements were recorded.

Because 32 individual experiments were performed, each steer was treated 1 to 3 times during the study. However, a minimum washout period of 21 days was implemented prior to any steer undergoing another experiment at a different dose. This washout period was based on results of experiments,[14] which indicated that the serum elimination half-life of MLA was 20.5 hours in cattle; thus, 99.2% (7 half-lives) of tall larkspur alkaloids were likely to be eliminated after 6 days. Therefore, after 21 days, any enzyme concentrations that may have been altered should have returned to typical values and the toxicokinetics of the subsequent experiments should have been similar to the first.

Physiologic monitoring—Assessors were not blinded to the dose or treatment administered. Heart rate was monitored by use of an established method.[15] Briefly, data were recorded with an instrument[b] equipped with an amplifier for signal amplification. Heart rate was monitored by use of repositionable monitoring electrodes[c] that were cemented in place with a gel-based formulation of a cyanoacrylate adhesive.[d] As described,[16] the leads were positioned with the positive electrode attached on the right scapula and the negative electrode attached on the sternum adjacent to the heart. A ground electrode was attached to the perineum. The heart rate signal was amplified with a gain range limited to ± 500 µV. The heart rate signal was filtered with a mains filter, a 60-Hz notch filter, a 120-Hz low-pass–0.1-Hz high-pass filter, and a digital band-pass filter with a high cutoff frequency of 45 Hz and a low cutoff frequency of 0.1 Hz. The cyclic measurements feature of the recording instrument[b] was used to calculate heart rate (beats/min). The heart rate in each steer was allowed to stabilize (typically during a 5-minute waiting period) prior to heart rate data collection. A 5-minute recording of heart rate was obtained immediately prior to administration of the single dose of the ground tall larkspur and 24 hours after dose administration.

Data analysis—Data are expressed as the mean ± SD for each physiologic response. Statistical comparisons between heart rate recordings at baseline and 24 hours were made by use of a 2-tailed, paired Student t test.[e] A value of $P < 0.05$ was indicative of a significant difference. The relationship between the ratio of MDL-type to MSAL-type alkaloid concentrations and the effective dose of MSAL-type alkaloids required to increase the heart rate in steers after oral gavage with finely ground tall larkspur was analyzed by plotting the MDL-type-to-MSAL-type alkaloid concentration ratio versus the effective dose of MSAL-type alkaloids. A linear regression analysis was performed on the plotted data points.[f]

Results

Electrospray mass spectra of alkaloid extracts from samples of the 3 tall larkspur populations were obtained (**Figure 1**). Deltaline (MH^+ m/z, 508) and MLA (MH^+ m/z, 683) were the 2 major norditerpenoid alkaloids in the *D barbeyi* extracts. The extract from *D glaucescens* primarily consisted of MLA. Samples from each population were analyzed for total alkaloid content and MSAL-type alkaloid content by use of an FTIR spectroscopy method.[12] The concentrations of the MDL-type, MSAL-type, and total alkaloids as well as the ratio of MDL-type to MSAL-type alkaloid concentrations for each tall larkspur population were calculated (**Table 1**). The concentrations of MSAL-type alkaloids in these collections were selected for calculation of the doses administered to the steers.

An effective dose was considered to be the amount of plant material that would significantly increase the heart rate and elicit clinical signs of toxicosis. The reference point for the effective dose at the start of the experiment was derived from the Manti collection (dose, 8 mg of MSAL-type alkaloids/kg); following administration of that dose, the heart rate increases and muscle weakness develops, but generally, the toxic effects do not cause cattle to become recumbent.[15] Treatment of 5 steers with plant material from the Manti collection (8 mg of MSAL-type alkaloids/kg dose) increased ($P = 0.041$) heart rate from 75 ± 9 beats/min at baseline to 102 ± 16 beats/min at 24 hours after dose administration (**Table 2**). A sixth steer was treated with plant material from the Manti collection; however, at the 24-hour time point, the steer was sternally recumbent. Therefore, heart rate analysis was not performed on the sixth steer. Treatment of 6 steers with plant material from the Cedar City collection (dose, 12 mg of MSAL-type alkaloids/kg) did not cause a change ($P = 0.057$) in the heart rate 24 hours after dose administration. The heart rate was 70 ± 5 beats/min at baseline and 82 ± 12 beats/min at 24 hours. Administration of a 13 mg of MSAL-type alkaloids/kg dose of plant material from the Cedar City collection was required to cause an increase ($P = 0.001$) in heart rate (baseline, 68 ± 14 beats/min; 24 hours, 100 ± 18 beats/min). Administration of a 12 mg of MSAL-type alkaloids/kg dose of plant material from the Dillon collection in 4 steers did not change heart rate ($P = 0.068$) 24 hours after treatment. The heart rate at baseline and 24 hours was 62 ± 10 beats/min and 73 ± 17 beats/min,

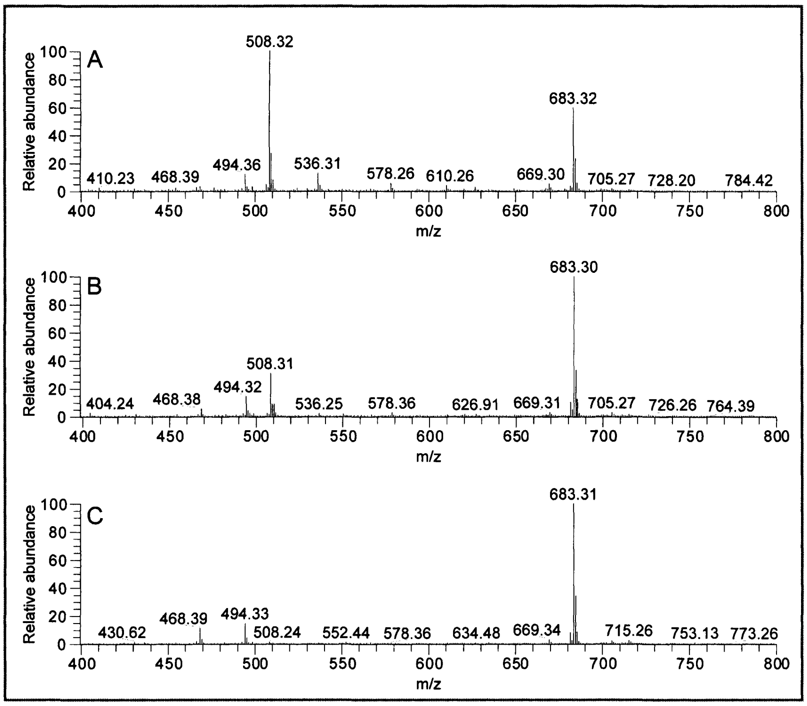

Figure 1—Representative electrospray mass spectrograms of the total alkaloid extract of 3 populations of tall larkspur from the Poisonous Plant Research Laboratory collection Nos. 03-12 (*Delphinium barbeyi* [Manti, Utah]; A), 07-06 (*D barbeyi* [Cedar City, Utah]; B), and 08-07 (*Delphinium glaucescens* [Dillon, Mont]; C). Specific spectrogram peaks include deltaline (MH^+ m/z, 508) and MLA (MH^+ m/z, 683).

Table 1—Results of FTIR spectroscopy analysis of norditerpenoid alkaloids extracted from finely ground preparations of dried plant material of 3 tall larkspur populations.

Tall larkspur population	MSAL-type (mg/g)	MDL-type (mg/g)	Total alkaloid (mg/g)	MDL-type-to-MSAL-type ratio
Delphinium barbeyi (Manti collection)	3.9	12.1	16.0	3.1:1
D barbeyi (Cedar City collection)	9.6	9.5	19.1	1.0:1
Delphinium glaucescens (Dillon collection)	8.2	5.1	13.4	0.6:1

The reported values represent the amount of alkaloid (mg) per gram of dried plant material. The dose of each tall larkspur population was administered on the basis of the MSAL-type alkaloid concentration. The tall larkspur population collections that are designated as Manti, Cedar City, and Dillon were collected from tall larkspur populations in Manti, Utah; Cedar City, Utah; and Dillon, Mont, respectively.

Table 2—Effects of single doses of 3 tall larkspur populations on heart rate in steers.

Tall larkspur population	Dose (mg of alkaloids/kg)			Heart rate (beats/min)*		
	MSAL-type	MDL-type	Total alkaloid	No. of steers	Baseline	24 hours
D barbeyi (Manti collection)	8	25	33	5	75 ± 9	102 ± 16†
D barbeyi (Cedar City collection)	12	12	24	6	70 ± 5	82 ± 12
	13	13	26	4	68 ± 14	100 ± 18†
D glaucescens (Dillon collection)	12	8	20	4	62 ± 10	73 ± 17
	14	9	23	3	61 ± 14	69 ± 5
	15	10	25	3	55 ± 3	74 ± 18
	18	11	29	4	65 ± 14	100 ± 21†

Steers were treated via oral gavage with varying amounts of 3 different tall larkspur populations. Heart rate was monitored immediately before (baseline) and at 24 hours after treatment. *Data represent the mean ± SD heart rate from 3 to 6 steers. †Value is significantly ($P < 0.05$) different from the baseline value. *See* Table 1 for remainder of key.

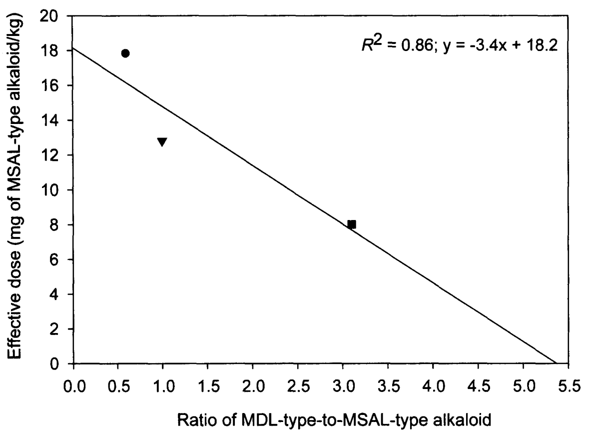

Figure 2—Linear regression relationship between the ratio of MDL-type to MSAL-type alkaloid concentrations and the effective dose of MSAL-type alkaloids (Manti collection [8 mg of MSAL-type alkaloids/kg; black square]; Cedar City collection [13 mg of MSAL-type alkaloids/kg; black triangle]; and Dillon collection [18 mg of MSAL-type alkaloids/kg; black circle]) required to increase the heart rate in steers after oral gavage with single doses of the dried and finely ground tall larkspur plant material. Each animal underwent 1 to 3 single-dose treatments. The MDL-type-to-MSAL-type alkaloid concentration ratio was plotted versus the dose of tall larkspur that was effective for increasing the heart rate of steers.

respectively. Increasing the dose of the MSAL-type alkaloids administered from the Dillon collection from 12 to 14 and 15 mg of MSAL-type alkaloids/kg did not alter heart rate ($P > 0.2$). An 18 mg of MSAL-type alkaloids/kg dose of plant material from the Dillon collection was required to increase heart rate from 65 ± 14 beats/min at baseline to 100 ± 21 beats/min at 24 hours ($P = 0.007$). An inverse relationship ($R^2 = 0.86$) was detected between the MDL-type-to-MSAL-type alkaloid concentration ratio and the dose (mg of MSAL-type alkaloids/kg) of tall larkspur plant material required to increase heart rate (**Figure 2**).

In addition to changes in heart rate, cattle were monitored for clinical signs of toxicosis (ie, decrease in gastrointestinal motility, shuffling gait, muscle weakness and trembling, or collapse). Following each dose administered for all 3 tall larkspur populations, there was an obvious visual change in fecal consistency, and steers typically defecated dry feces 24 hours after treatment. However, the cattle had no detectable signs of constipation until the dose administered also caused an increase in heart rate. The steers treated with plant material from the Manti collection (8 mg of MSAL-type alkaloids/kg) had signs of weak muscular tremors in the head and shoulder regions 7 hours after dose administration. Muscle weakness was even more pronounced 24 hours after treatment, and 1 of 6 steers was sternally recumbent. The steers treated with plant material from the Cedar City collection (12 mg of MSAL-type alkaloids/kg) did not have detectable signs of toxicosis; however, when treated with 13 mg of MSAL-type alkaloids/kg, steers had detectable signs of toxicosis, including muscle tremors, shuffling gait, and dry feces. The steers treated with plant material from the Dillon collection did not have any clinical signs of toxicosis until a dose of 15 mg of MSAL-type alkaloids/kg was administered; however, the clinical signs detected at this dose were very minor. Steers treated with an 18 mg of MSAL-type alkaloids/kg dose of plant material from the Dillon plant population had overt clinical signs, and 2 of the 6 steers were sternally recumbent 24 hours after treatment.

Discussion

Results of previous research[5,6] have indicated that the MSAL-type alkaloids are more toxic than the MDL-type alkaloids. Consequently, management recommendations[9,10] for grazing cattle on ranges that contain toxic tall larkspur are based primarily on the concentration of MSAL-type alkaloids in tall larkspur. However, the MDL-type alkaloids are generally more abundant in many species of tall larkspur.[1,7] Until recently, it was not clear whether a high concentration of MDL-type alkaloids in tall larkspur plants increases the toxicity of tall larkspur plants or whether that toxicity is solely attributable to the MSAL-type alkaloids. Data obtained from research[11] in mice suggest that MDL-type alkaloids enhance the overall acute toxic effects of MLA in an additive manner.

In the present study, steers were treated with ground plant material collected from 3 populations of tall larkspur that had different concentrations of MDL- and MSAL-type alkaloids. We selected ground plant material for 2 reasons. First, this reduced the difficulty of isolating and purifying sufficient amounts of pure norditerpenoid alkaloids for administration to cattle. Second, the administration of plant material is more similar to situations encountered during grazing that would be associated with tall larkspur toxicosis than the administration of alkaloid extracts.

Three different populations of tall larkspur, which were known to contain a wide spectrum of MDL-type-to-MSAL-type alkaloid concentration ratios, were selected. We hypothesized that MDL-type alkaloids affect the toxicity of the tall larkspur plant material in an additive manner. Thus, a tall larkspur population with high MDL-type alkaloid concentration would be more toxic than a tall larkspur population with low MDL-type alkaloid concentration, given similar MSAL-type alkaloid content. Results from the present study indicated that as the ratio of MDL-type to MSAL-type alkaloid concentrations decreased, the amount of plant material required to increase heart rate also increased. A decrease in the MDL-type to MSAL-type alkaloid concentration ratio from 3.1:1 to 0.6:1 required the dose to be increased from 8 to 18 mg of MSAL-type alkaloids/kg to induce an increase in heart rate. Coincidentally, the dose that increased heart rate was > 26 mg of total alkaloids/kg for each tall larkspur population. Consequently, it could be argued that exceeding a threshold concentration of total alkaloid is all that is required to increase heart rate. However, we detected[14] a positive correlation (Spearman r = 0.75) between the increase in heart rate associated with tall larkspur poisoning and serum MLA concentration ($P < 0.001$) but not serum deltaline concentration ($P = 0.2$). Additionally, a dose of 37.6 mg of total alkaloids/kg derived from a tall larkspur population that almost exclusively contains MDL-type alkaloids did not cause an elevation in heart rate (unpublished data). These results are similar to findings of a study[11] in mice and suggest that MDL-type alkaloids increase the toxicity of tall larkspur plants by potentiating the toxicity of the MSAL-type alkaloids.

Although higher concentrations of MSAL-type alkaloids were required to elicit clinical signs in steers treated with tall larkspur containing reduced concentrations of MDL-type alkaloids, the difference in the total amount of plant material administered was small and well within the quantity that a cow could consume while grazing in a rangeland setting. The amount of dried plant material required for an effective dose of the Manti collection was 960 ± 41 g; for the Cedar City and Dillon collections, the amounts were 665 ± 13 g and 1,066 ± 49 g, respectively. This indicates the Cedar City collection would be the most toxic of these 3 populations on the basis of the amount of plant material required to provide an effective dose. This was expected because the Cedar City collection had the highest concentration of MSAL-type alkaloids and total alkaloids of the 3 populations. Interestingly, on the basis of previous assumptions that only the MSAL-type alkaloids affect the toxicity of tall larkspur plants, the Dillon collection would presumably be the second most toxic population. However, we demonstrated that the Manti collection was more toxic than the Dillon collection. Therefore, even though the concentration of the MSAL-type alkaloids are the most important factor, the results from the present study suggest that the MDL-type alkaloids play an important role in the toxicity of tall larkspur by potentiating the toxicity of the MSAL-type alkaloids.

It is noteworthy that the effect of the MDL-type alkaloids appeared to be more pronounced in steers than in mice. In mice,[11] a change in the ratio of MDL-type to MSAL-type alkaloid concentrations from 1:1 to 5:1 resulted in a 7% change in the LD_{50}. However, in the present study, a change in the ratio of MDL-type-to-MSAL-type alkaloid concentrations from 1:1 to 3:1 resulted in 63% difference in the dose, on the basis of the MSAL-type alkaloid content required to provide an effective dose. There are several possible reasons for the differences between these 2 studies, including selection of dissimilar endpoints (eg, a lethal dose vs an effective dose); potential difference in the nicotinic acetylcholine receptors between steers and mice, which could result in the MDL-type alkaloids being more toxic in steers than in mice; differing effects of IV administration of purified compounds versus oral administration of ground plant material; and potential differences in the metabolism and subsequent toxicokinetic profile of norditerpenoid alkaloids in steers and mice. The data from a study[11] in mice suggest that the MDL-type alkaloids have no effect on the elimination of MLA. However, it may be possible that large quantities of MDL-type alkaloids hinder the elimination of the MSAL-type alkaloids in cattle, thereby increasing the bioavailability of the more toxic MSAL-type alkaloids and effectively increasing the toxic potential of the plant material. Results of another study[14] indicated that an increase in heart rate in cattle poisoned with tall larkspur species is directly correlated with serum MLA concentration and not with serum deltaline concentration. Additional studies are needed to determine whether the elimination of MSAL-type alkaloids from the 3 populations of tall larkspur differs.

One note of caution to consider when making management recommendations on the basis of the results of the present study is that the cattle were treated with a single bolus dose of ground plant material. A single bolus dose of ground plant material does not accurately represent the conditions under which cattle are poisoned by tall larkspur species when grazing on a range. One study[17] revealed that there are 3 distinct thresholds associated with tall larkspur toxicosis: a subclinical toxicosis that results in reduced tall larkspur consumption for 1 to 3 days but no overt signs nor overall reductions in consumption of other forage, a short-acting toxicosis with overt clinical signs that results in reduced food intake for several days but no long-term effects, and a potentially fatal toxicosis with severe clinical signs that may result in death. The authors of that study[17] postulated that cyclic consumption of larkspur enables cattle to generally regulate tall larkspur consumption below the second threshold in a typical range setting, which allows most cattle the opportunity to utilize larkspur as an otherwise nutritious plant. Consequently, additional studies involving various dosing regimens and forms of larkspur that more realistically mimic grazing situations need to be conducted before final recommendations are made.

Results from the present study indicated that the MDL-type alkaloids contribute to the toxicity of MSAL-type alkaloid–containing tall larkspur plants in cattle. However, the MSAL-type alkaloids, such as MLA, are more toxic than MDL-type alkaloids and as such are the primary factors responsible for the toxicity of larkspur plants. Therefore, consumption and absorption of a sufficient quantity of MSAL-type alkaloids is required for a tall larkspur plant to be toxic. However, MDL-type alkaloids appear to potentiate the overall toxicity of the MSAL-type alkaloids and should be considered when predicting the potential toxicity of tall larkspur populations. Therefore, when chemical analyses are performed on tall larkspur to assess the toxic potential, the concentrations of both the MSAL-type and total alkaloids should be determined, with more consideration given to the MSAL-type alkaloids. The results from this study indicated that tall larkspur plants containing large amounts of MDL-type alkaloids, in addition to high MSAL-type alkaloid content, should be considered potentially more dangerous to cattle than plants with only high MSAL-type alkaloid content.

a. Gehl Mix-All, model 55, Gehl Co, West Bend, Wis.
b. Octal Bioamp, ADInstruments Inc, Colorado Springs, Colo.
c. 3M Red Dot, model 2670, 3M Corp, Saint Paul, Minn.
d. Henkel Consumer Adhesive Inc, Avon, Ohio.
e. SigmaStat for Windows, version 3.1, SPSS Inc, Point Richmond, Calif.
f. SigmaPlot for Windows, version 9.0, SPSS Inc, Point Richmond, Calif.

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